

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

James E. GALEN

Appln. No.: 09/993,292

Confirmation No.: 5386

Filed: November 23, 2001

For: USE OF ClyA HEMOLYSIN FOR EXCRETION OF PROTEINS



Docket No: A8461

Group Art Unit: 1645

Examiner: Duffy, P.

SUBMISSION OF EXECUTED DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Submitted herewith is a copy of an executed Declaration Under 37 C.F.R. §1.132 signed
by James E. GALEN.

Respectfully submitted,

A handwritten signature of Drew Hissong.

Drew Hissong
Registration No. 44,765

SUGHRUE MION, PLLC
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

WASHINGTON OFFICE
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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Docket No: A8461

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SECOND DECLARATION OF JAMES E. GALEN UNDER 37 C.F.R. §1.132

Commissioner for Patents

P.O. Box 1450

Alexandria VA 22313-1450

Sir:

I, James R. Galen, hereby declare and state:

THAT I am a citizen of the United States of America;

THAT I have received the degree of Ph.D. in 1991 from the University of Maryland

Baltimore;

THAT I have been employed by the Center for Vaccine Development since 1993, where I hold a position as Associate Professor, with responsibility for engineering expression systems for attenuated *Salmonella enterica* serovar Typhi human live vector vaccine strains.

Comments regarding inclusion of sequences in application

Paragraph 0028 of U.S. application number 09/993,292 refers to four polynucleotide sequences encoding bacterial proteins by name and GENBANK accession number. Namely:

The polynucleotide encoding *Shigella flexneri* truncated hlyE - Accession No. AF200955

The polynucleotide encoding *Escherichia coli* hlyE - Accession No. AJ001829

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The polynucleotide encoding *Salmonella paratyphi clyA* - Accession No. AJ313033

The polynucleotide encoding *S. Typhi clyA* - Accession No. AJ313034

Each of the four polynucleotides was submitted to GENBANK and assigned an accession number prior to the date on which the present application was filed. None of the polynucleotide sequences is known to have been changed in the period of time between the filing of the present application (November 23, 2001), and the date of execution of the instant Declaration.

Furthermore, based on reasonable investigation, there is no evidence that any of the sequences have been changed during this period of time. Indeed, as shown on the attached GENBANK printouts for each of the four sequences (Appendix I-IV), there were no changes to the sequences under the relevant accession numbers since the filing date (November 23, 2001) of the pending application. With regard to AF200955, the last date of amendment was February 2, 2000 (Appendix D). With regard to AJ001829, the last date of amendment was April 2, 1998 (Appendix II).

With regard to AJ313033, the last date of amendment was April 15, 2005 (Appendix III). However, as shown on the enclosed summary of amendments made to the listing (Appendix V), the only changes that have been made are those to the classification of the organism, the references citing the accession number, and the addition of a SwissPro reference number (please see the boxed regions on Appendix V). No changes have been made to the sequence.

With regard to AJ313034, the last date of amendment was April 15, 2005 (Appendix IV). However, as shown on the enclosed summary of amendments made to the listing (Appendix VI), the only changes that have been made are those to the classification of the organism, the

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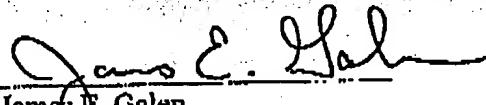
references citing the accession number, and the addition of a SwissPro reference number (please see the boxed regions on Appendix VI). No changes have been made to the sequence.

As such, I aver that the reference material referred to herein contains no new matter and consists of the same material incorporated by reference into the above-identified U.S. patent application at the time the application was filed.

* * *

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 7/11/05


James E. Galen

APPENDIX I



Nucleotide

Nucleotide

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Range: from to Reverse complemented strand Features: SNP CDD MGC
 HPRD STS

□ 1: AF200955. Reports *Shigella flexneri*...[gi:6478772]

Links

LOCUS AF200955 904 bp DNA linear BCT 03-FEB-2000
 DEFINITION Shigella flexneri HlyE (hlyE) gene, complete cds.
 ACCESSION AF200955
 VERSION AF200955.1 GI:6478772
 KEYWORDS .
 SOURCE Shigella flexneri
 ORGANISM Shigella flexneri
 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
 Enterobacteriaceae; Shigella.
 REFERENCE 1 (bases 1 to 904)
 AUTHORS Wallace,A.J., Stillman,T.J., Atkins,A., Jamieson,S.J.,
 Bullough,P.A., Green,J. and Artymiuk,P.J.
 TITLE E. coli hemolysin E (HlyE, ClyA, SheA): X-ray crystal structure of
 the toxin and observation of membrane pores by electron microscopy
 JOURNAL Cell 100 (2), 265-276 (2000)
 PUBMED 10660049
 REFERENCE 2 (bases 1 to 904)
 AUTHORS Green,J.
 TITLE HlyE of Shigella flexneri
 JOURNAL Unpublished
 REFERENCE 3 (bases 1 to 904)
 AUTHORS Green,J.
 TITLE Direct Submission
 JOURNAL Submitted (02-NOV-1999) MBB, University of Sheffield, Western Bank,
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APPENDIX II



Nucleotide

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ANSWER **ANSWER** **ANSWER** **ANSWER**

Range: from to Reverse complemented strand Features: SNP CDD MGC
 HPRD STS

□ 1: AJ001829. Reports *Escherichia coli* ...[gi:3021363]

Links

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 DEFINITION Escherichia coli clyA gene.
 ACCESSION AJ001829
 VERSION AJ001829.1 GI:3021363
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 SOURCE Escherichia coli
 ORGANISM Escherichia coli
 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
 Enterobacteriaceae; Escherichia.
 REFERENCE 1
 AUTHORS Ludwig,A., Bauer,S., Benz,R. and Goebel,W.
 TITLE Molecular analysis of a latent pore-forming 34-kDa haemolysin from
 Escherichia coli K-12
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 1080)
 AUTHORS Ludwig,A.
 TITLE Direct Submission
 JOURNAL Submitted (30-SEP-1997) Ludwig A., Lehrstuhl fuer Mikrobiologie,
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 Hubland, D-97074 Wuerzburg, GERMANY
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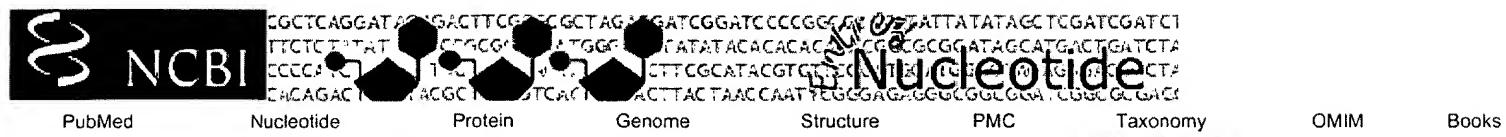
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Range: from to Reverse complemented strand Features: SNP CDD MGC
 HPRD STS

[1]: AJ313034. Reports Salmonella typhi ...[gi:14018374]

Links

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 VERSION AJ313034.1 GI:14018374
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 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
 Enterobacteriaceae; Salmonella.
 REFERENCE 1
 AUTHORS Oscarsson, J.
 JOURNAL Thesis (1999) Department of Microbiology, Umea University, Umea,
 Sweden
 REFERENCE 2
 AUTHORS Oscarsson, J., Westermark, M., Lofdahl, S. and Uhlin, B.
 TITLE Expression of a pore-forming cytotoxin by Salmonella ser. Typhi and
Salmonella ser. Paratyphi A
 JOURNAL Unpublished
 REFERENCE 3 (bases 1 to 1102)
 AUTHORS Oscarsson, J.
 TITLE Direct Submission
 JOURNAL Submitted (07-MAY-2001) Oscarsson J., Molecular Biology, Umea
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REFERENCE

AUTHORS

TITLE

JOURNAL

REFERENCE

AUTHORS

TITLE

JOURNAL

REFERENCE

AUTHORS

TITLE

JOURNAL

FEATURES

[SKIP: unchanged/identical feature/sequence/publication information is not displayed]

Protein reviews on the web

<http://www.ncbi.nlm.nih.gov/entrez/sutils/girevhist.cgi?gene=clyA>

Sequence Revision History

		Protein	Genome	Structure	PMC	Taxonomy	OIMM	Books
Show difference in		<input type="checkbox"/> GenBank/GenPept	<input checked="" type="checkbox"/> [AJ313033]	<input type="checkbox"/> format	<input type="checkbox"/> Show	<input type="checkbox"/> Update Date	<input type="checkbox"/> Go	<input type="checkbox"/> Clear
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14140227			1			May 17 2001 1:25 AM		

LOCUS	SEN313033	1102 bp	DNA	linear	BCT	15-APR-2005
LOCUS	SEN313033	1102 bp	DNA	linear	BCT	15-MAY-2001
DEFINITION	Salmonella paratyphi clyA gene for cytolsin A.					
ACCESSION	AJ313033					
VERSION	AJ313033.1	GI:14140227				
KEYWORDS	Salmonella paratyphi	(Salmonella enterica subsp. enterica serovar Paratyphi A)				
SOURCE	Salmonella paratyphi					
ORGANISM	Salmonella paratyphi					
REFERENCE	1	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Salmonella.				
REFERENCE	1	Bacteria; Proteobacteria; gamma subdivision; Enterobacteriaceae; Salmonella.				
REFERENCE	1	(bases 1 to 1102)				
AUTHORS	Oscarsson, J.					
JOURNAL	Thesis (1999)	Department of Microbiology, Umea University, Umea, Sweden				
REFERENCE	2					
REFERENCE	2	(bases 1 to 1102)				
AUTHORS	Oscarsson, J., Westermark, M., Lofdahl, S. and Uhlin, B.					
TITLE	Expression of a pore-forming cytotoxin by Salmonella ser. Typhi and Salmonella ser. Paratyphi A					
JOURNAL	Unpublished					
REFERENCE	3	(bases 1 to 1102)				
AUTHORS	Oscarsson, J.					
TITLE	Direct Submission					
JOURNAL	Submitted (07-MAY-2001) Oscarsson J., Molecular Biology, Umea University, Umea, 90187, SWEDEN					
FEATURES	Location/Qualifiers					

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ORIGIN

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APPENDIX VII



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2

(bases 1 to 1102)

2

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3

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3

Reference sequence project

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FEATURES

[SKIP: unchanged/identical feature/sequence/publication information is not displayed]

Clusters of orthologous groups

Protein reviews on the web

gene

75..986

/gene="clyA"

75..986

/gene="clyA"

Gi	Version	Update Date
14018374	1	Apr 16 2005 12:40 AM
14018374	1	May 11 2001 2:43 PM

LOCUS	SEN313034	1102 bp	DNA	linear	BCT 15-APR-2005
LOCUS	SEN313034	1102 bp	DNA	linear	BCT 09-MAY-2001
DEFINITION	Salmonella typhi clyA gene for cytolysin A.				
ACCESSION	AJ313034				
VERSION	AJ313034.1	GI:14018374			
KEYWORDS	clyA gene; cytolysin A.				
SOURCE	Salmonella enterica subsp. enterica serovar Typhi				
ORGANISM	Salmonella enterica subsp. enterica serovar Typhi				
	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;				
REFERENCE	Enterobacteriaceae; Salmonella.				
REFERENCE	1	Bacteria; Proteobacteria; gamma subdivision; Enterobacteriaceae;			
REFERENCE	1	Salmonella.			
AUTHORS	Oscarsson, J.				
JOURNAL	Thesis (1999) Department of Microbiology, Umea University, Umea, Sweden				
REFERENCE	2				
AUTHORS	Oscarsson, J., Westermark, M., Lofdahl, S. and Uhlin, B.				
TITLE	Expression of a pore-forming cytotoxin by Salmonella ser. Typhi and Salmonella ser. Paratyphi A				
JOURNAL	Unpublished				
REFERENCE	3	(bases 1 to 1102)			
AUTHORS	Oscarsson, J.				
TITLE	Direct Submission				
JOURNAL	Submitted (07-MAY-2001) Oscarsson J., Molecular Biology, Umea University, Umea, 90187, SWEDEN				
FEATURES	Location/Qualifiers				

Clusters of orthologous groups	gene	75..986
Protein reviews on the web	CDS	75..986
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/product="cytolysin A"
/protein_id="CAC38363_1."
/db_xref="GI:14018375"
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ORIGIN

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121	cgcgcgaaac	cgcgcgatgg	gcattatgatc	tttataacaa	ataccctcgac

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